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POLYEMBRYONY, HETEROZYGOSIS AND CHIMERAS IN CITRUS[1]

HOWARD B. FROST

INTRODUCTION

The genus Citrus is characterized by remarkable genetic variability, both in seed reproduction and within clonal varieties. An F_1 hybrid progeny usually exhibits great genetic diversity (Swingle, 1913*a*), suggesting the F_2 generation from a cross between races differing in many genes. Bud-variation types arise frequently, and involve changes in many characters of tree and fruit (Shamel *et al.*, 1918, 1918*b*, 1920).

With any tree fruit, considerations of time and expense seem to make thorough genetic analysis impracticable; with Citrus, polyembryony appears to render it even theoretically impossible. Such remarkable genetic phenomena, however, occurring in a group of such great economic importance, deserve the best interpretation possible on the basis of the theory of heredity worked out with organisms more suitable for genetic study.

The present paper reports evidence, obtained from the pedigree cultures of Citrus at the Citrus Experiment Station, bearing on the relation of apogamy to genetic variation in Citrus. It also includes preliminary data which can best be presented in a general publication preceding detailed reports on limited problems. A comprehensive review of the literature of Citrus genetics is not attempted,

[1] Paper No. 135, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

but the principal published evidence is briefly discussed, and an attempt is made to evaluate its general significance and to indicate clearly the outstanding problems.

The various Citrus forms mentioned in this paper will usually be designated by their ordinary English names. These names, with the corresponding Latin names according to Swingle (1914, 1914*a*, 1915) except for the species recently discussed by Merrill and Lee (1924), are as follows: kumquat, *Fortunella* spp.; lemon, *C. Limonia* Osbeck; *mandarin, tangerine, etc., C. nobilis and varieties (King mandarin* or orange, *C. nobilis* Lour.; Willow-Leaf or China mandarin and Dancy tangerine, *C. nobilis* var. *deliciosa* Swingle; satsuma, *C. nobilis* var. *Unshiu* Swingle); sweet orange (often called simply "orange" in this paper), *C. sinensis* Osbeck; sour orange, *C. Aurantium* L.; pummelo (including grapefruit and shaddock), *C. maxima* (Burm.) Merrill. The citranges are hybrids between *C. sinensis* and trifoliate orange (*Poncirus* (*Citrus*) *trifoliata* Raf.).

Since the "Satsuma orange," or "Satsuma mandarin," is classed by Swingle as a distinct botanical variety, and includes several horticultural varieties (Scott, 1919), the name *satsuma* is here used as a common noun, synonymous with the Japanese *unshiu*. There seems to be no good English group name for the King type.

In accordance with Swingle (1914*a*, 1916) and Merrill and Lee (1924), *grapefruit* is used for the type of *C. maxima* (var. *uvacarpa* Merrill and Lee) commonly cultivated in America. The general American public has not accepted *pomelo* as the name of this fruit, and the authors just cited show that its acceptance is not desirable, since *pummelo* (Swingle, 1916), spelled in various ways, is widely used in other countries for the shaddocks and forms intermediate between shaddocks and grapefruit. Since it is very convenient to have one English name that is applicable to the whole species, and *pummelo* is in use for a great range of types, while *grapefruit* and *shaddock* are definitely established in the United States and the West Indies to designate extreme forms, it seems desirable to include under the term *pummelo* all forms belonging to the species *Citrus maxima* (*C. grandis* Osbeck). Swingle (1916, p. 2751), in one brief reference, seems to advocate this delimitation of the term, although elsewhere he (1914*a*, 1916) excludes the grapefruit, which is little grown in the Oriental regions where the name *pummelo* is in general use. The somewhat extensive use of *pomelo* for the grapefruit in American horticultural literature is a further reason for applying *pummelo* to the whole species, instead of excluding the grapefruit. This appli-

cation of the term is of decided advantage in horticultural naming of hybrids of grapefruit; *tangelo* (from *tangerine* and *pomelo*) is well established in horticultural literature, and such forms as *lemelo* and *mandelo* are likely to be needed.

In the following discussion of Citrus genetics, *it is concluded, from the available evidence, that Citrus forms are in general extremely heterozygous, as the Hagedoorns (1914) have suggested. This conception seems highly significant in relation to various aspects of Citrus genetics. In the evolution of this heterozygosis, polyembryony probably was an important factor.*

I do not wish to appear, however, to prejudge the case, either in general or with reference to any particular instance or type of variation here considered. Certain alternative explanations and possible objections will therefore be mentioned at this point.

Some of the variations are certainly very remarkable; for example, Swingle's (1913*a*) lemon-*trifoliata* hybrids which bear hypophylls instead of normal leaves, and his citranges bearing many leaves with five leaflets. The different combinations in F_1 hybrids from one cross (as the citranges of Webber and Swingle), of various parental characters, even of ones that seem to be respectively characteristic of the parent species, suggest the variability of dominance which Swingle has inferred to exist. It may be that trees with extra chromosomes occur frequently among the sexual progeny[2] generally, as it appears that tetraploid trees do among the apogamic progeny in our cultures. This or some other exceptional or little known cause may account for some of the striking variations among hybrids. "Zygotaxis," the hypothesis proposed by Swingle (1913*a*) as a possible general explanation of the great variability of Citrus hybrids, is especially discussed below, in the section on "Heterozygosis."

Recent work on Oenothera cytology (S. H. Emerson, 1924) indicates that chromosome behavior in that genus differs widely from the Drosophila type which seems so widely prevalent. In a few of our parent varieties which have been examined cytologically, however, it appears that the chromosomes usually pair and separate normally at meiosis in the pollen mother cells, and as a rule produce normal-appearing pollen tetrads. It is therefore improbable that a majority of their hybrids have aberrant chromosome numbers, unless chromosome elimination after fertilization is very common in hybrids. The

[2] In the interest of conciseness, the terms *apogamic* and *sexual* will frequently be used in this paper, combined with such words as *embryo, seedling,* and *progeny,* in the sense of "produced by apogamy" and "produced by fertilization," respectively.

high variability of F_1 Citrus hybrids is, however, a very general phenomenon, not limited to a minority of the individuals. Further, enough is known, in general, of the production of new characters by new combinations of genes in crossing, to warn us against setting any narrow limits to the probable results of recombination in crosses between two highly heterozygous species. On the other hand, a new warning against undue confidence in gene stability is given by Eyster's (1924) recent hypothesis of qualitative division of certain genes. Possibly genes specially affecting other characters are sometimes unstable in the same way as are certain genes relating to variegation. If Citrus is especially notable for the occurrence of such unstable genes, this fact may account for part of the remarkable variability that is observed.

POLYEMBRYONY

Citrus seeds are frequently polyembryonic. Strasburger (1878, 1907) showed that the supernumerary embryos are formed by proliferation of nucellar cells surrounding the embryo sac. These adventitious embryos may be expected, therefore, to reproduce the seed-parent genotype, without variation due to segregation in sporogenesis or to recombination in fertilization.

Citrus polyembryony is not entirely due to nucellar budding, however, for in 10 (probably 11) cases in our cultures, among more than 1000 hybrids, two hybrid seedlings have come from one seed. The seeds were planted separately, and all operations on which the reliability of the pedigrees depended were so carefully performed and checked that the single-seed origin of the pairs of seedlings is beyond doubt. The budding and consequent labeling were done with similar care. In eight of the ten cases, both of the original seedlings, as well as trees budded from them, have been positively classified as hybrids; in the ninth case, both of the small seedling trees are almost surely hybrids, as the budded trees certainly are; and in the tenth case one seedling died undescribed, so that its record depends on budded trees alone. In the eleventh case, one of the two "seedlings" died young, and their separateness below the surface of the soil was not proved. Since in every case the two hybrids seem to be identical in type, in spite of the usual great diversity among hybrids of the same parentage, it is probable that these are all cases of "identical twins," each

pair being derived from one fertilized egg. The seed-parent varieties that have produced identical-twin hybrids are: King, Owari satsuma and Willow-Leaf (*C. nobilis*); Ruby and Valencia (*C. sinensis*); and Imperial (*C. maxima*). Part of the pollen parents are indicated in footnote *a* to table 2; those concerned in the three cases from earlier cultures were Dancy and Willow-Leaf.

Less than one per cent of our hybrid-producing seeds have given two hybrids each. Since the apogamic progeny from crossing (recognized by their strictly maternal characters) have been nearly three times as numerous as the hybrids, it is plain that the fission or budding of sexually produced embryos plays only a minor part in the total production of supernumerary embryos. Since the number of apogamic embryos per seed is indefinite, occasional fission in apogamic embryos may occur, but could be detected only by microscopic examination.

As is shown by the data of table 1, Citrus seeds are highly variable in number of embryos. The embryos examined were highly variable in size and often irregular in shape. Some were very small, and possibly some smaller ones escaped observation.

There must be, therefore, much opportunity for competition among embryos within Citrus seeds, and it may be that many are eliminated at early stages of development. There is plainly much opportunity for the fertilized egg to be crowded out by apogamic embryos. The chances of such elimination must depend largely on the number of adventitious embryos that start, and on the position and the relative age and vigor of the two classes of embryos. Comparison of table 1 with the *Total seedlings* column of table 2 indicates that very many of the apogamic embryos fail to germinate. Germination must therefore give much opportunity for selective elimination; survival may be determined by differences in size, vigor, position, morphological completeness, and susceptibility to infection. Albinism (pp. 377–379) causes the early death of many of the seedlings from some parents.

Citrus presents therefore one form of the "developmental selection" (natural selection acting within the soma of the parent) whose evolutionary significance has been discussed by Buchholz (1922). Many of the embryos from fertilized eggs must compete with apogamic embryos in the same seed, and with such embryos in other seeds of the same fruit. Genotypes inferior in vigor to the seed parent must be more severely handicapped in Citrus than in forms where competition is between ordinary monoembryonic seeds. On the other hand, viable sexual embryos are entirely unnecessary for reproduction, provided apogamic embryos are able to develop. The added difficulty

TABLE 1

NUMBER OF EMBRYOS PER SEED IN TEN SEEDS OF EACH OF TEN CITRUS VARIETIES

	Frequency (a)												Mean embryos per seed
Embryos:	1	2	3	4	5	6	7	8	9	10	11	12	
King mandarin(*b*)	10												1.0
Sweet lemon(*b*)	9(*e*)	1(*d*)											1.1
Lisbon lemon(*b*)	8(*e*)	2(*e,f*)											1.2
Eureka lemon(*b*)	8	1(*d,e*)	1(*d*)										1.3
Ruby (blood) orange(*c*)	3(*e*)	5	1	1									2.0
Mediterranean Sweet orange(*c*)	3	4(*d*)	2				1						2.4
Dancy tangerine(*c*)	3	1	4		1	1							2.8
Imperial grapefruit(*c*)	1	1	2(*e*)	3(*e*)	1		1	1					4.1
Same, *fruit No. 6(2)* (*c,g*)	4(*d*)	3	3										1.9
Owari satsuma(*c*)			1		6(*d*)			2	1(*d*)				5.8
Willow-Leaf mandarin(*c*)			2		2	2	1	1(*d*)		1(*d*)		1	6.5

(*a*) A few doubtful *decisions* are included without special indication. Except as noted, the embryos had two cotyledons each.

(*b*) Random samples, each from mixed seed of ten fruits of the crop of 1925, from open pollination; dissection and *description* by A. C. Austin.

(*c*) Random samples, *each* (with the *one listed exception*) from mixed seed of several fruits of the crop of 1917; dissection and description by M. H. Roblee. Satsuma seeds from open pollination, the rest from selfed (bagged) flowers.

(*d*) In each case one embryo with only one cotyledon.

(*e*) In *each* case, one embryo with three cotyledons.

(*f*) One embryo with four cotyledons.

(*g*) A fruit with remarkably short, small seeds.

in Citrus breeding which results from the occurrence of apogamic embryos has been pointed out by Webber (1900).

It seems, however, that apogamic embryos do not often develop in the absence of fertilization. Strasburger (1907) states that fertilization precedes the formation of adventitious embryos, and that, while the latter are usually present, the sexually produced embryo is seldom absent. Webber (1905) reports that seeds have occasionally resulted from flowers protected from pollination, but considers that fertilization is usually a prerequisite for seed formation. In ordinary solid plantings of the Washington navel orange, a variety which, according to Osawa (1912), produces a few good embryo sacs but no pollen at all, seeds are very rare; yet, in my work and elsewhere (Coit, 1915), fruits from artificially pollinated flowers of this variety have very often contained seeds. I have obtained similar results with a variety of satsuma (evidently Owari; Scott, 1919) which from its usual seedlessness, the appearance of its pollen, and Osawa's (1912) cytological study of the "unshiu" appears to have little or no functional pollen. Out of 79 satsuma fruits from artificial cross-pollination by "seedy" varieties, 66 contained seeds, while 34 fruits from flowers bagged for selfing on the same trees in the same two seasons were all entirely seedless. The fruits produced by varieties with good pollen, similarly bagged for selfing, usually contained seeds. Other observations agree with these.

Altogether, it seems very probable that Citrus seeds do not often develop without pollination, although seedless fruits sometimes develop without pollination even in varieties normally seedy. In view of the abundant production of adventitious embryos, this fact is somewhat surprising. It would appear (Strasburger, 1907; Webber, 1905) that the nucellar budding which produces the adventitious embryos is at least very largely dependent on some growth stimulus due to the fertilized egg. Although certain species hybrids, such as many citranges (Swingle, 1910) and the Sampson tangelo, seem to give apogamic progeny exclusively when selfed, this fact does not demonstrate their ability to form apogamic embryos without fertilization.

In crosses between species, where the hybrids can usually be positively distinguished from the apogamic progeny, we may expect (see p. 369) to find a negative correlation between the percentage of hybrids and the amount of apogamy characteristic of the seed-parent species—and also between percentage of hybrids and characteristic vigor of apogamic seedlings.

TABLE 2

NUMBERS OF TOTAL SEEDLINGS PER SEED AND OF HYBRID SEEDLINGS PER SEED, FROM INTERSPECIFIC CROSSES

Crosses involving the same seed parent combined. (*a*)

Seed-parent variety(*b*)	Number of pollen-parent varieties(*b*)	Number of seeds giving seedlings	Total seedlings (per cent of seeds)	Hybrid seedlings (per cent of seeds) (*c*)
Sweet lemon	2	22	100	100
Lisbon lemon	2	62	106	83.9±3.1
King mandarin	5	332	100.6	79.8±1.5
Eureka lemon	2	119	108	73.9±2.7
Mediterranean Sweet orange	3	105	110	61.0±3.2
Ruby orange	1	42	119	47.6±5.2
Imperial grapefruit	5	503	128	46.7±1.5
Valencia orange	2	57	135	28.1±4.0
Owari satsuma	3	193	139	21.2±2.0
Willow-Leaf mandarin	5	714	127	18.6±1.0
Dancy tangerine	2	54	126	18.5±3.6
Navel oranges (2)	2	55	136	7.3±2.4

(*a*) This table includes only classified progeny; the actual germination, and probably the number of seedlings per seed, were greater, since a large number of young seedlings died from various causes, including albinism. In seven certain cases and one probable case of the production of two hybrids from one seed, the seeds concerned are omitted from the tabulation. There were two cases in series 120 (see table 3 for parentage of series), and one each in series 24, 72, 100, 104 (the separateness of the two "plants" was not proved), 107 and 119.

(*b*) Including all in the cultures of 1917, excepting the cases of intraspecific crosses.

(*c*) The probable error is obtained from $.67449\sqrt{\frac{pq}{n}}$, where p is the observed percentage of hybrids, q is $1-p$, and n is the corresponding total number of seeds giving classified seedlings.

Table 2 gives data bearing mainly on the former point, for all the available series of the hybrid cultures of 1917,[3] arranged in the order of the percentage of hybrids. When we consider both the data of table 1 and the actual "percentage of seedlings," it is evident that viable hybrids tend to become scarcer as embryos become more numerous. The two varieties most conspicuously polyembryonic in table 1 are among the lowest in number of hybrids in table 2. On the other hand, the five varieties that are lowest in embryos are

[3] All lots are included except those from intraspecific crosses. There are a few cases of doubtful classification, but it is very improbable that the final results will make much change. The classification has been made by tree characters throughout, but it has already been confirmed in very many cases, and rarely corrected, on examination of fruit.

highest in hybrids. The differences in hybrids between these two groups of seed parents are, in general, highly significant statistically. The former group produced a high percentage of seedlings, and the latter group a low percentage, thus confirming the indications of table 1 as to characteristic numbers of embryos.

With the intermediate varieties of table 1, the correlation is much less regular, but the hybrid percentages are in most cases intermediate between those of the groups just discussed. Dancy tangerine (embryos medium) and the navel oranges (no embryo count) have both given a high seedling percentage (see also Coit, 1915, p. 58) and a low hybrid percentage; in both cases the young apogamic seedlings are decidedly vigorous, and competition may be especially severe in proportion to the number of apogamic embryos present. The most marked exception to the general trend of the results is Imperial grapefruit, which has a rather high hybrid percentage in spite of its rather high embryo count, its high seedling percentage, and the decided vigor of its apogamic seedlings; this variety is, however, definitely intermediate between the two groups first mentioned in both embryos and hybrids.

Mediterranean Sweet seems (table 1) to produce a considerable number of apogamic embryos when selfed, yet it has given a low seedling percentage and a high hybrid percentage. This may be a result of its relatively low vigor of growth, as shown both by orchard trees and by young apogamic seedlings. All the other orange varieties of table 2 have produced more numerous and more vigorous apogamic seedlings, and a smaller percentage of hybrids.

The promptness with which apogamic development begins after fertilization may differ in different varieties, and differences in this respect may affect the proportion of viable fertilized eggs.

The negative correlation between total seedlings and hybrid seedlings shown by the tables may be due in part, of course, to more extensive elimination of *apogamic* embryos in lots where the sexual embryos are especially vigorous in comparison. Thus the recorded medium embryo count for Mediterranean Sweet may be fairly representative for selfing, but not for crossing. It should be noted, however, that in the orchard the hybrid progeny of this seed parent are frequently somewhat *less* vigorous than the apogamic progeny.

There remains the general question of how far the proportion of hybrids may be affected by the pollen parents. Table 3, which segregates the data of table 2 by pollen parents, suggests an approach to random-sampling variation among the lots from each seed parent,

TABLE 3

NUMBER OF TOTAL SEEDLINGS PER SEED AND OF HYBRID SEEDLINGS PER SEED

Data of table 2, progeny of each cross given separately. (*a*)

Seed-parent variety (clone)	Series	Pollen-parent variety (clone)	Number of seeds giving seedlings	Total seedlings (per cent of seeds)	Hybrid seedlings (per cent of seeds) (*b*)
Lisbon lemon	91	Valencia orange	25	100	88.0±4.4
	92	*Imperial grapefruit*	*37*	*111*	*81.1±4.3*
King mandarin	97	Genoa lemon	18	106	72.2±7.1
	98	Lisbon lemon	4	(100)	(100)
	99	Mediterranean Sweet orange	166	100	85.5±1.8
	6	Valencia orange	35	103	60.0±5.6
	100	Imperial grapefruit	109	100	78.0±2.7
Eureka lemon	89	*Valencia orange*	*37*	114	62.2±5.4
	90	Imperial grapefruit	82	105	79.3±3.0
Mediterranean Sweet orange	113	King mandarin	36	111	63.9±5.4
	114	Willow-Leaf mandarin	50	112	54.0±4.8
	116	Imperial grapefruit	19	105	73.7±6.8
Ruby orange	72	Dancy tangerine	42	119	47.6±5.2
Imperial grapefruit	117	"Hedge bergamot" (*c*)	108	111	54.6±3.2
	118	Eureka lemon	6	(117)	(17)
	119	*Lisbon lemon*	73	121	63.0±3.8
	120	Willow-Leaf mandarin	270	136	41.9±2.0
	121	Orange (blood, tree N102)	46	137	34.8±4.7
Valencia orange	22	Dancy tangerine	21	138	9.5±4.3
	24	Willow-Leaf mandarin	36	133	38.9±5.5
Owari satsuma	101	Lisbon lemon	2	(150)	(50)
	54	Valencia orange	82	139	20.7±3.0
	102	Imperial grapefruit	109	139	21.1±2.6
Willow-Leaf mandarin	103	Lisbon lemon	6	(117)	(17)
	104	Ruby orange	172	124	21.5±2.1
	105	Valencia orange	192	128	12.0±1.6
	106	Orange (blood, tree N102)	59	131	30.5±4.0
	107	Imperial grapefruit	285	128	18.9±1.6
Dancy tangerine	95	"Hedge bergamot" (*c*)	7	(114)	(0)
	96	Imperial grapefruit	47	128	21.3±4.0
Washington orange	108	Willow-Leaf mandarin	17	141	5.9±3.9
Orange (navel, tree N1).	110	Dancy tangerine	6	(133)	(17)
	111	Willow-Leaf mandarin	32	134	6.25±2.9

(*a*) The 22 progeny of Sweet lemon, omitted here, were from pollination by Mediterranean Sweet orange (7 trees) and Imperial grapefruit.

(*b*) The probable error is obtained as in table 2.

(*c*) *A peculiar form with brachytic shoots, occasionally used for hedges in California. It is* very unlike typical *Citrus bergamia* Risso, and may be closer to sour orange; it resembles the form which Risso and Poiteau (1818–22) described under the name *C. bigaradia crispifolia*.

although some of the differences appear statistically significant. This table shows clearly that the more significant differences of table 2 are not due to differences in fertility or viability with different pollen parents. This fact is especially well shown by comparison of Imperial with all other pollen parents (table 3). With four seed parents, Imperial alone (table 3) has given hybrid percentages very similar to those given by the combined pollen parents (table 2); and with Eureka and Mediterranean Sweet the differences between pollen parents are less than three times their probable error. On the other hand, the cross Imperial by Willow-Leaf has given more than twice the hybrid percentage of the reciprocal cross, and the difference is about nine times its probable error.

Even in the case of differences between pollen parents that appear statistically significant (none are unequivocally so), the indications as to differential fertility or viability are very dubious, because of the probability of non-random differences between fruits in the percentage of hybrids. That is, the variability of the percentage of hybrids from any cross, among the lots of seedlings produced by the respective seed-parent fruits, may tend to be greater than is to be expected from the general percentage of hybrids among the total progeny from the cross in question. Such a situation may exist, if the physiological conditions favoring apogamy, within a given seed-parent variety, vary markedly by whole fruits or branches, since in this case the variability, in number of embryos, *of the seeds taken by single-fruit lots,* will tend to be greater (Fisher, chap. 10) than if the fruits were substantially random samples of seeds from one statistical population for amount of apogamy. Such high variability among fruits in amount of embryonic competition would be expected to give high variability in the percentage of viable hybrids. In this case, wide differences in hybrid percentage, in different crosses involving the same seed parent, would be less significant than if the fruit lots of seeds were random samples with respect to viable hybrid embryos.

Statistical study of the variability of the percentage of hybrids is needed, but must be deferred until the records of the numbers of hybrids have been completely checked and revised on the basis of fruit characters. Some of the records are so suggestive, however, as to justify mention of the hypothesis just stated. An especially striking illustration may be added. With Willow-Leaf as seed parent, the hybrids are often so grouped with reference to the parent fruits as to suggest that the variation in embryonic competition among the

fruits was by no means random. For instance, series 104 gave a total of 37 hybrids from 172 seeds, but three relatively few-seeded fruits from one bagged branch gave 10 hybrids from 13 seeds, and one other fruit gave (excluding the case of identical-twin hybrids) 6 hybrids from 8 seeds. Thus the other 151 seeds reported in table 3 gave only 21 hybrids. The fruits mentioned gave a total-seedling percentage of only 100, while the other 151 seeds gave a percentage of 128. Evidently those four fruits had seeds with relatively few apogamic embryos, in which the hybrids encountered little competition. Table 1 mentions an Imperial fruit whose seeds were small and had comparatively few embryos.

With each of the first four seed parents of table 3, the crosses higher in total seedlings are generally lower in hybrids. The numbers are evidently too small, however, to make these differences significant even when taken together. Imperial shows similar differences, except that the two lots relatively low in seedlings show the reverse difference in hybrids between themselves. Satsuma shows practically no difference between pollen parents. The differences in hybrids with Willow-Leaf, with relatively large numbers, show no definite relation to the differences in total seedlings.

It is probably significant in this connection, that King and the lemons, which have few extra embryos, have rather frequently given weak hybrids when used as seed parents in interspecific crosses, while with satsuma and Willow-Leaf, which have many embryos, weak hybrids seem decidedly less common. In the latter case, presumably, the severity of the apogamic competition seldom permits weak hybrid embryos to survive.

It may fairly be concluded that the differences in the percentage of hybrids depend mainly on the seed parents.

We therefore have experimental evidence indicating that Citrus varieties differ greatly in abundance of apogamic embryos, and that, in varieties which produce relatively numerous apogamic embryos, the embryo resulting from fertilization is relatively often crowded out. There is also some indication of a negative correlation between vigor of apogamic embryos and percentage of viable sexual progeny. These considerations have an important bearing on breeding procedure. Varieties which produce relatively few adventitious embryos can be more economically used as seed parents. Counts of embryos often give valuable indications in this connection.

VARIATION AMONG SEEDLINGS PRODUCED BY APOGAMY

In our pedigreed cultures, albino and partially albinistic seedlings have occurred in various lots of progeny, both from selfing and from crossing, and sometimes in surprising abundance. In some cases (fig. 1) two such seedlings came from the same seed, so it is very probable that at least one of these, in each case, was produced by

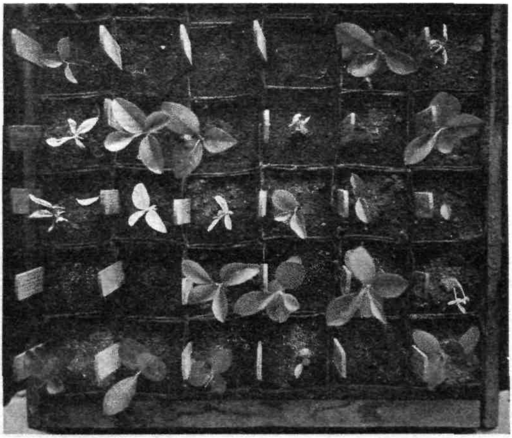

Fig. 1. Albinos among F_1 seedlings from the cross Paper-Rind (St. Michael) orange ♀ × Imperial grapefruit ♂, about 12 weeks after sowing. The albino leaves are small and in some cases already withering.

apogamy. In many cases the same seed produced both albinistic and green seedlings. Some seedlings are light green; others are partly green and partly white, but these often seem to lack the definite delimitation of ordinary variegation. Apparently no seedlings long survive unless they produce fully green leaves at a very early stage. No cases of true variegation have been observed among older seedlings.

The large proportion of albinistic seedlings which may occur is illustrated by table 4. Reason will be given below for expecting

higher proportions of apogamic progeny from selfing than are reported above for crossing.[4] In view of all the facts stated, it is very improbable that the albinistic seedlings are all or mainly extracted recessives *carrying the same gene for albinism*. Probably, in fact, many or most of them are produced apogamically. If this is the case, their abundance is very remarkable, especially when we consider the rarity of albinism and chlorophyll variegation as observed bud variations on orchard trees.

TABLE 4

ALBINISM IN A STOCK SEED-BED, FROM SEEDS OF ORDINARY ORCHARD FRUITS (*a*)

Type of seedling	Grapefruit		Sweet orange		Sour orange	
	Number	Per cent of total(*b*)	Number	Per cent of total	Number	Per cent of total
Green	176		173		237	
Whitish	16	7.4±1.2	16	7.2±1.2	4	1.6
White	24	11.1±1.4	33	14.9±1.6	4	1.6
All albinistic	40	18.5±1.8	49	22.1±1.9	8	3.3
Undetermined(*c*)	4		1		3	
Total	216		222		245	

(*a*) Blocks of seedlings systematically selected to avoid prejudice.

(*b*) The probable error is obtained as in table 2. With two exceptions, it is omitted with percentages under 10.

(*c*) Omitted from totals.

An infectious type of variegation, such as occurs in Abutilon (Babcock and Clausen, 1918, p. 381), is improbable in the present case, since the parent trees and (usually) the majority of the progeny are fully green. The albinism seems to be genetic, not pathological.

It may be worth while to suggest a provisional hypothesis for albinism. The recent demonstration by Demerec (1923) and Lindstrom (1924) of numerous genes for albinism in maize, together with the evidence for extensive heterozygosis in Citrus discussed below, suggests the possibility of high proportions of albinos among the sexual progeny. Further, somatic gene mutation in a tree heterozygous for albinism genes might often produce islands of albinistic tissue, and any embryo developed from these areas, either apogamically or sexually, would be albinistic. The great objection to the latter possibility is the scarcity of visible albinistic areas in older

[4] The consideration of the evidence (p. 388) for this expectation necessarily ignores the albinistic plants, which die before any other character than albinism can be determined.

trees. Bateson (1919, 1921), however, has found that certain green-over-white periclinal chimeras do not give reversals of the relative position of their components, although the corresponding white-over-green chimeras do give such reversal; he suggests that differences in growth vigor may be concerned in this result. Possibly albinism often originates as a somatic variation in Citrus, although albinistic areas rarely develop far enough to be noticeable. It must be noted here that permanent green-and-white forms, evidently chimeras, do occur (Shamel *et al.*, 1920), and appear to have a mixed or mosaic condition of the apical meristem.

We must keep in mind here the aberrant genetic phenomena so often associated with variegation (R. A. Emerson, 1922; Eyster, 1924).

Since the albinism is often only partial, and variegation so often shows genetic peculiarities, we cannot safely conclude that other characters are likely to be similarly variable among apogamic seedlings. Webber (1905), however, has reported remarkable variability among apogamic seedlings from interspecific crossing, finding in one case "5 or 6 different varieties" among 20 non-hybrid F_1 grapefruit seedlings produced by one cross. He concluded that such apogamic progeny appeared more variable than seedlings resulting from selfing. These variations seem to have related mainly to fruit characters.

Among the apogamic progeny from cross pollination that are now under observation at Riverside, genetic variations recognizable *in advance of fruiting*, aside from the "thick-leaved" form discussed below, and the proable case of albinism, seem to be very rare. The doubtful point here is the uncertainty whether a few variant individuals in our cultures are apogamic or not, but the evidence from crosses between very unlike forms indicates that these variants are usually hybrids, and therefore favors the interpretation just stated. On the other hand, among the apogamic progeny whose fruits have so far been studied in our cultures, several apparent cases of genetic variation have been observed. In the best-substantiated case, an old navel orange tree (N1 of table 3; not Washington), pollinated by grapefruit, has given apogamic progeny mostly with navel-marked fruit, usually seedless, and flowers destitute of pollen, but also including several trees that produce flowers with pollen and non-navel fruits with seeds. In general, so far, the apogamic progeny from selfing and those from crossing appear to be identical in type.

Genetic variation among apogamic progeny does not necessarily indicate the immediate agency of genic or chromosomal mutation,

since the seed-parent tree may often be in a chimeral condition as the result of earlier genetic changes in somatic tissue.

It must be noted that the possibility of frequent variation among the apogamic embryos prevents strictly positive conclusions as to the proportion of sexual embryos, especially from selfing, and as to the genetic variability of the sexual embryos.

In this discussion, exception has been made of a thick-leaved form, showing no pollen-parent characters in crosses, which has frequently appeared in our cultures. This form, readily identifiable everywhere

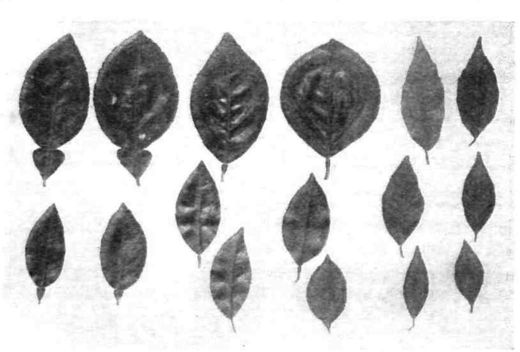

Fig. 2. Each vertical row of two or three typical leaves (one large, from a vigorous shoot, above, and one or two smaller below) represents a tree budded from a seedling. The leaves shown here and in the following figures, except as noted, were taken from trees that had grown two or three summers in the orchard. Figure 2 includes apogamic progeny only. First vertical row at left: seed-parent type from cross-pollination of Marsh grapefruit ("seedy strain"). Second row: thick-leaved type from same cross. Third and fourth rows: seed-parent and thick-leaved types, both from the same seed, from cross-pollination of Ruby orange. Fifth and sixth rows: the same for Willow-Leaf mandarin, both types from one seed.

by the same general characteristics, has been found among the apogamic progeny of four horticultural varieties of sweet orange, three of grapefruit, four of *Citrus nobilis* (King, Dancy, Willow-Leaf, and Owari satsuma, and one of lemon. It often constitutes several per cent of the total number of progeny. As compared with ordinary apogamic seedlings of the same parentage, it is characterized by broad, thick leaves, stout shoots and thorns, somewhat lower vigor and slowness to bloom. Figure 2 shows, for three parent varieties, the differences in leaf form between normal and thick-leaved apogamic

progeny of the same parentage. Figures 3 and 6 give the same comparison for the thick-leaved and normal apogamic forms of Imperial grapefruit, as produced both by crossing and by selfing.

I have examined fruits from thick-leaved lemon (Lisbon), and a few from thick-leaved tangerine (Dancy), mandarin (Willow-Leaf) and orange (Paper-Rind and Ruby). In all these forms the oil glands of the rind appear larger than in the corresponding diploid apogamic progeny, and the surface of the rind has a characteristically coarser appearance. The acid content of the juice seems generally lower than in diploids. The lemon fruits seem approximately normal in juiciness and flavor, but the actual yield of juice in three tests was very low. The Dancy, Willow-Leaf, and Paper-Rind fruits were notably inferior in texture or flavor, or in both respects.

A doubled number of chromosomes ($n = 18$) has been reported for a thick-leaved form of orange (Frost, 1925*a*), and the thick-leaved form of grapefruit shown in figure 2 has recently been found to be tetraploid. Presumably, therefore, the other ten thick-leaved forms are also tetraploid. Since a thick-leaved and a normal apogamic seedling often arise from the same seed, it is probable that tetraploidy originates frequently, under the Riverside climatic conditions, in the nucellar tissue of Citrus species generally.

Muller (1925) has recently indicated the probable reasons why polyploid races originate much more readily in plants than in animals. Citrus seems to offer, in its development of embryos from single cells of somatic tissue, the most favorable general conditions possible for the origin of tetraploid individuals. Presumably tetraploid forms of Citrus have usually been eliminated, however, under both natural and artificial selection, by unfavorable tree and fruit characters. There probably has been little opportunity, therefore, for natural production of triploids from tetraploids, and seed reproduction of triploids would doubtless be hampered by a high degree of gametic sterility.

A few of our hybrids have characters suggesting triploidy. Presumably triploids and modified triploids can be produced by crossing tetraploids with diploids. If the horticultural disadvantages of tetraploids are generally absent from triploids, the production of triploids may become an important aspect of Citrus breeding, for several reasons. Triploids may be expected to be practically seedless. They *might* prove especially vigorous. There might also be advantage in the possibility of using a double dose of one parent type in the production of hybrids. And hybrid tetraploids should permit the production of triploid hybrids having, *on the average*, equal chromo-

some contributions from three ancestral races. Very frequent irregularities of chromosome reduction, as observed in pollen mother cells of thick-leaved orange (Frost, 1925*a*), may interfere seriously with the production of triploids.

Especially interesting breeding possibilities are suggested by Clausen and Goodspeed's (1925) tetraploid Nicotiana. This form, although derived from a sterile F_1 species hybrid having irregular meiosis, is fertile, evidently because meiotic pairing occurs, in the tetraploid, only between chromosomes derived from the same ancestral species, with consequent *complete homozygosis* and normal reduction. If interspecific Citrus hybrids will produce, asexually, tetraploids behaving in this way, these tetraploids should produce (aside from cytological accidents and new gene mutations) *gametes that are all alike*. Most of the progeny produced sexually by selfing such a tetraploid would presumably be indistinguishable from the progeny produced apogamically. In crossing, however, the possibility of using a hybrid *as a homozygous parent* might prove very useful. Even if the other parent were a highly heterozygous diploid, the number of possible F_1 types would be enormously reduced as compared with that resulting from the crossing of two such diploids.

Even with normal random reduction in a pure-species tetraploid, the recessive genes for which the plant is heterozygous should be largely "covered" by corresponding dominants in its gametes. These gametes should, therefore, represent the parental type much more closely, as a rule, than the gametes of the diploid form from which the tetraploid arose. If, however, that diploid form was highly heterozygous, the tetraploid may have more meiotic pairing between its *identical* chromosomes than between its *non-identical homologous* chromosomes. The normally produced gametes would then tend to represent their parent still more closely, the limiting case being the complete homozygosis that prevails when pairing regularly occurs between identical chromosomes.

This largely speculative discussion of the breeding possibilities of tetraploids seems to be justified by the slowness with which genetic data are obtained in Citrus, and the consequent especial desirability of formulating the problems with great care. The horticultural prospects of tetraploids seem to depend mainly on three factors: (1) the extent to which the tetraploid condition, and perhaps the triploid also, are in themselves inimical to desirable tree and fruit characters; (2) the meiotic behavior of tetraploids; (3) the possibility of obtaining tetraploids, especially homozygous ones, from diploid species hybrids.

HETEROZYGOSIS

Webber (1900*a*, 1905, 1906, 1907, 1912), Swingle (1910, 1913, 1913a) and Webber and Swingle (1905) have described and discussed the remarkable variability of F_1 species hybrids in Citrus. The salient features of the case may be stated as follows: (1) Various species of Citrus cross readily with each other and also (evidently less readily) with species of Poncirus (trifoliate orange) and Fortunella (kumquat); (2) these species usually seem to give, on selfing, only a

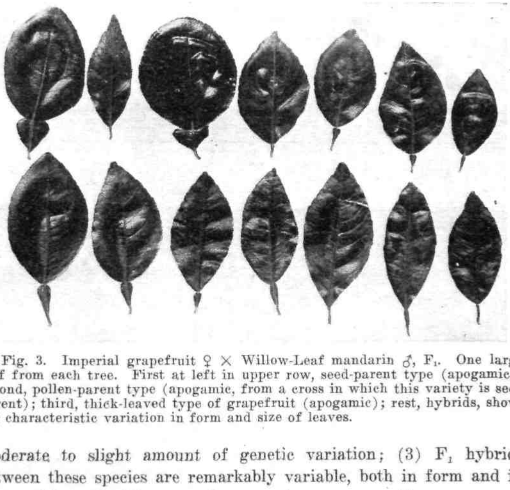

Fig. 3. Imperial grapefruit ♀ × Willow-Leaf mandarin ♂, F_1. One large leaf from each tree. First at left in upper row, seed-parent type (apogamic); second, pollen-parent type (apogamic, from a cross in which this variety is seed parent); third, thick-leaved type of grapefruit (apogamic); rest, hybrids, showing characteristic variation in form and size of leaves.

moderate to slight amount of genetic variation; (3) F_1 hybrids between these species are remarkably variable, both in form and in vigor; (4) in the F_2 generation conspicuous segregation may occur, or the F_1 form may apparently breed true. Figures 3 and 4 illustrate the variability in size and form of leaves among the F_1 hybrid progeny from two species crosses, and figure 5 the variability in size and form of fruit in another cross.

As Swingle (1913*a*) especially emphasizes, the F_1 hybrids may appear far more variable than either uncrossed parent species. To

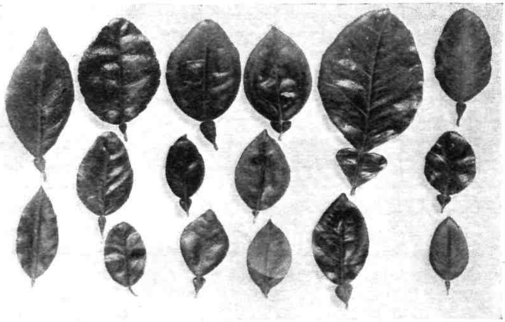

Fig. 4. Arrangement as in figure 2. Mediterranean Sweet orange ♀ × Imperial grapefruit ♂, F_1. Seed-parent type at left, the rest hybrids. (The pollen-parent type is shown in figure 3.)

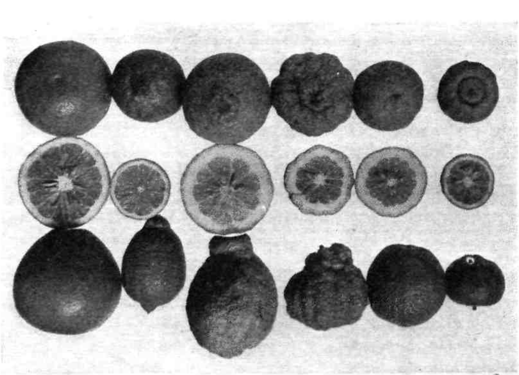

Fig. 5. Fruits, each vertical row from one tree. Imperial grapefruit ♀ × Lisbon lemon ♂. From left to right: seed-parent type, pollen-parent type (from reciprocal cross), and four hybrids.

explain this situation, he proposes the hypothesis of *zygotaxis*, which assumes "a positional or vectorial influence of chromosomes." That is, it is supposed that the relative effect of the individual chromosomes upon ontogeny depends to a large extent on their relative position in the nucleus, and that this position normally changes only at the time of fertilization. Adequate cytological support for this hypothesis is lacking, however, and the results of genetic experimentation seem unfavorable.

Sturtevant (1925) has recently secured from Drosophila certain evidence which seems to indicate that position may affect the potency of a gene. He concludes that two mutant genes of the bar series have more effect on development when they are carried in the *same* chromosome (as in double-bar, formerly called "ultrabar") than when they are in *homologous* chromosomes (as in homozygous bar). Another case, involving triploids, is similarly interpreted, the conclusion as to position of the genes depending in part on the fact that homologous chromosomes are "closely apposed" in somatic divisions.

Muller (1918) concludes that extensive heterozygosis in a pair of chromosomes may be expected to decrease their mutual attraction, and so to favor abnormalities of meiosis. Such abnormalities are often observed in hybrids from wide crosses. As Swingle (1913*a*) suggests, if the homologous chromosomes of the progeny of closely related parents tend to be associated in pairs in the somatic nuclei, that association may well be weakened or destroyed in interspecific hybrids.

The considerations stated in the last two paragraphs may be held to give some basis for the hypothesis of zygotaxis. At best, however, it seems seriously inadequate as the main explanation of the Citrus phenomena in question. If a pairing attraction is weakened in species hybrids, there is no obvious reason, cytological or genetic, to expect that many definite and distinct chromosome configurations, permanent throughout somatic life and with extremely marked effects on the relative potency of genes, will be established. The *accidental* formation of somatically permanent chromosome configurations at fertilization seems especially improbable; therefore, if many such different *permanent* configurations should occur among the zygotes from one cross, it would seem that they must depend on genic differences among the gametes which united to form those zygotes—or, in other words, *on extensive heterozygosis of the parents.* If, however, the parents are thus heterozygous, the genic differences among the progeny will

probably account directly for at least the major portion of their somatic variability.

Such cases as that of beaded wing in Drosophila (Muller, 1918) show that recessive genes may often be suppressed by linkage with other genes which have a recessive lethal effect, and that selfing or inbreeding may fall far short of revealing all the genetic potentialities of an organism, as represented by its genic constitution. It is also generally conceded that the effect of a particular gene may be greatly modified by differences in other pairs of allelomorphs. It is now evident that heterozygosis offers almost unlimited possibilities of F_1 variability, and therefore the hypothesis of zygotaxis seems to meet no serious general need in genetic theory.

It may fairly be assumed, therefore, that the striking variability among F_1 Citrus hybrids is mainly due to heterozygosis of the parent forms. Evidence bearing on this hypothesis will now be considered. Variation in chromosome number may be concerned in some cases, but this seems very improbable as a regular source of such extensive series of forms.[5]

A. C. and A. L. Hagedoorn (1914) have suggested that Citrus varieties are highly heterozygous but self-sterile, and that when not cross-pollinated they reproduce by apogamy alone. The evidence now indicates, however, that self-sterility is not concerned, and that the *viable* progeny from selfing, although more largely of apogamic origin than in the case of crossing, are often not exclusively so.

Coit (1914) states that the evidence indicates that cross-pollination is unnecessary "in all naturally fertile varieties of orange." Ikeda (1904) reports that cross-pollination between certain varieties of orange results in failure to set fruit. My observations indicate that, in Citrus varieties with good pollen, seeds are set about as readily in selfing as in crossing. That this situation is not usually due to apogamy in the absence of fertilization, is indicated by evidence already presented (p. 371).

There is also direct evidence for the occurrence of segregation with selfing. Swingle (1910) reports that some F_1 citranges "reproduce almost exactly the parental type" in their progeny, while with other citranges part or all of the progeny show typical F_2 variability, ranging nearly from one P_1 species to the other. Evidently the *viable* embryos are all produced apogamically in the first group, but not in

[5] Longley (1925) has recently found that two Citrus hybrids, one of them intergeneric according to Swingle's (1914) classification, have the normal number of chromosomes ($n = 9$).

the second. Similar evidence from selfing of commercial varieties of Citrus has been obtained in our cultures. As a specific illustration, among 122 seedlings from 99 seeds of selfed Imperial grapefruit, 13 young trees show marked differences from the usual type of the variety (fig. 6); 7 of these belong to the thick-leaved type and are doubtless apogamic in origin (p. 380), but the 6 others probably represent as many distinct recombination types.

When F_1 cultures from selfing and from crossing are compared, there is in general a remarkable parallelism. We commonly find (aside from the thick-leaved type) two very distinct groups of

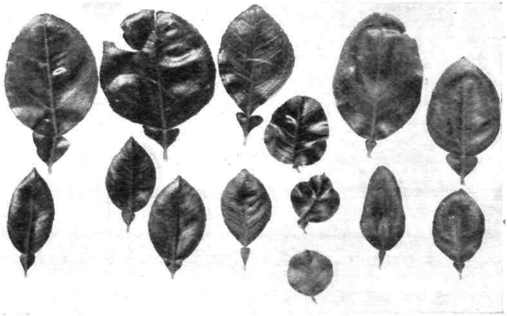

Fig. 6. Arrangement as in figure 2. Progeny of selfed Imperial grapefruit. The three vertical rows at the right are from two-year trees in nursery rows. *First at left, seed-parent type; the rest, variant types (second, thick-leaved type, from same seed as first tree; fourth, a small, weak type).*

progeny, which occur in interspecific crosses, in intraspecific crosses (as between two varieties of sweet orange), and with selfing. One group consists of trees essentially identical with the seed-parent variety, with occasional differences such as Webber (1905) has discussed, while the other group, often relatively small, consists of marked variants. In the case of crossing, the former group plainly is entirely of apogamic origin, while trees of the latter group usually show clearly the influence of the pollen parent. That the former group is mainly or entirely of apogamic origin in the case of selfing also, seems highly probable; if not, the parent trees must usually breed true to a very remarkable extent with self-fertilization, while producing extremely wide variation with cross-fertilization.

We have only about 600 progeny from selfing; none of these are in the cultures of 1917 which give our best hybrid data, and very few of them come from the parent varieties with few adventitious embryos. The available evidence is therefore meager. It strongly suggests, however, that sexually produced progeny are generally rarer with selfing than with crossing, and much less vigorous. Lisbon lemon has given 9 progeny from selfing, of which 4 appear to be identical with the apogamic progeny from crossing. The other 5 progeny are all markedly variant, and range from trees considerably inferior to Lisbon in vigor to ones so feeble that it is difficult to keep them alive. It seems plain that the latter group corresponds to the hybrids in the cultures from crossing. Similarly, selfed King, among 38 progeny from 38 seeds, has given 29 trees all evidently typical King, 1 thick-leaved, and 8 other variants. In this case fruits from nearly all the trees that closely resemble the parent variety have been examined, and these trees all seem to be identical in type with apogamic progeny from cross-pollination of King. The variants from selfed King, besides being much less numerous than the King-like progeny, are all or nearly all inferior in vigor (at least 6 of the 8); on the other hand, the hybrids in the corresponding lots from cross-pollination constitute more than half of the total progeny, and are usually similar to their apogamic sibs in vigor. The progeny of selfed Imperial have already been discussed. Part of the other selfed varieties (Paper-Rind orange, Ruby, Willow-Leaf) have given (besides occasional "thick-leaved" progeny) a very small proportion of conspicuous variants, always weak, and several varieties (some in very small cultures) seem to have produced apogamic progeny alone.

In the causation of inviability, albinism (pp. 377–379) may be of much importance.

Evidently, then, ordinary Citrus varieties, as well as many F_1 hybrids (Swingle, 1910) reproduce mainly or very largely by apogamy when selfed. Selfing probably produces, as a rule, fewer and weaker viable sexual progeny than does crossing. This situation has an important bearing on the variability of stock seedlings (Webber, 1920). Probably most of the undesirable variant types among nursery seedlings are produced by fertilization. Citrus clones which give genetically uniform seedlings from selfing are evidently not ones which "breed true" in the ordinary sense, but ones which reproduce almost entirely by apogamy. From this point of view, clones which produce seeds with fairly numerous embryos are likely to give better results than clones with usually monoembryonic seeds. The suitability

of the Florida Rough lemon for use as a stock plainly depends partly on the fact that it is highly polyembryonic, and therefore, unlike the Lisbon lemon, reproduces mainly by apogamy when selfed.

The F_1 hybrids from a Citrus cross often vary greatly in vigor, as well as in morphological characters (fig. 7). Forms conspicuously lacking in vigor are often produced. Crandall (1922) has reported similar results with interspecific crosses of apples, and Dr. M. J. Dorsey, on examining some of our Citrus hybrids recently, stated that they seemed less variable than hybrids between certain plum species. Wellington (1924) has reported that numerous varietal crosses of apples have usually produced some weak types among the F_1 progeny. He ascribes this result, together with the great variability of fruit characters, to extensive heterozygosis of the parent clones. Where *all the hybrids are feeble*, as in some apple crosses, it may be inferred that the genetic reaction systems of the parent species are *in general* too unlike to permit normal development in their hybrids (Goodspeed and Clausen, 1917). When, however, some F_1 hybrids are vigorous and some feeble, it may be inferred that the parents are heterozygous and that *some progeny combinations of genes* are markedly more favorable than others. With maize (Jones, 1918) the unfavorable combinations usually seem to be homozygous recessives, since selfing usually decreases vigor and increases the proportion of abnormal recessive types. In view of the predominance of unfavorable recessives among the mutations of Drosophila (Muller, 1918), and the probability (Sturtevant, 1921) "that closely related species have many genes in common," the unfavorable combinations in species crosses of Citrus may be, in large part, merely cases of absence of various favorable dominant genes that are heterozygous in the parents.

In the apple and the plum, extensive heterozygosis is favored by widespread self-sterility, and also, if mutation occurs in somatic cells, by the long life of the individual; it is therefore to be expected that crosses, whether varietal or specific, will give highly variable F_1 populations.

In Citrus, self-sterility does not seem to be concerned, but the long-life factor[6] is present and the frequent bud variations suggest, although they do not demonstrate (Clausen and Goodspeed, 1923) the occurrence of gene mutation. The conditions therefore seem to be favorable (Muller, 1918; Jones, 1918) for the accumulation of unfavorable recessive genes. Moreover, the long-life factor is markedly

[6] The word *factor* is used in its general sense in this paper; in the sense of "genetic factor," *gene* is employed exclusively.

reënforced by apogamy. A Citrus race might be heterozygous for many recessive genes that are sublethal or lethal when homozygous, and yet reproduce by apogamy for an indefinite period.

The conditions in Citrus are also favorable for the development of heterozygosis by crossing, since the flowers are attractive to insects and produce an abundance of pollen, while (among the true Citrus species) there seems to be no interspecific sterility to impose limitations on wide crossing.

The evidence on heterosis[7] in Citrus is also in accord with the assumption of extensive heterozygosis. It seems probable that ordinary Citrus varieties are complex heterozygotes, whose unfavorable recessive genes cause a great and general decrease of vigor on selfing, and frequent decrease of vigor even in species crosses.

In our cultures, hybrids decidedly exceeding in vigor the more vigorous parental type, as represented by its apogamic progeny, seem to be exceptional, while feeble, slow-growing hybrid forms are common in some crosses (fig. 7). Marked heterosis, such as Webber and Swingle (1905) report for some of the citranges, seems at first sight to be unusual in these crosses. It must be noted, however, that apogamic embryos do not furnish a satisfactory standard of comparison for the estimation of heterosis in hybrids; the proper standard is obviously given by the sexual embryos produced by selfing.

It has been shown that sexually produced progeny seem to be decidedly more numerous with crossing than with selfing. This conclusion agrees with the expectation that cross-fertilization will produce the more vigorous embryos, more often able to withstand the competition of those produced by apogamy.

It seems significant that Swingle (1910) finds such marked vigor, even as compared with the parental forms, in citranges, which are to be considered intergeneric rather than interspecific hybrids; as would be expected, F_2 citranges and back crosses with the orange are less vigorous. Further, Swingle reports that crosses of F_1 citranges with the grapefruit, which belongs to a species markedly different from the orange, or with the kumquat, representing a third genus, yield still more vigorous progeny. Thus it appears that as a rule the hybrids from the widest crosses are decidedly the most vigorous. If we could adequately compare hybrids between and within species of true Citrus (as Swingle delimits the genus) with sexually produced progeny from selfing of the same species and clones, presumably we should find

[7] The theory of heterosis, or hybrid vigor, proposed by Jones (1918) is here accepted as the best general explanation of this phenomenon.

much heterosis even in these hybrids, and far more evidence of selective elimination with selfing than with crossing. In general, we may conclude that, with Citrus and its near relatives, the expression of unfavorable genes among the progeny decreases with distance of

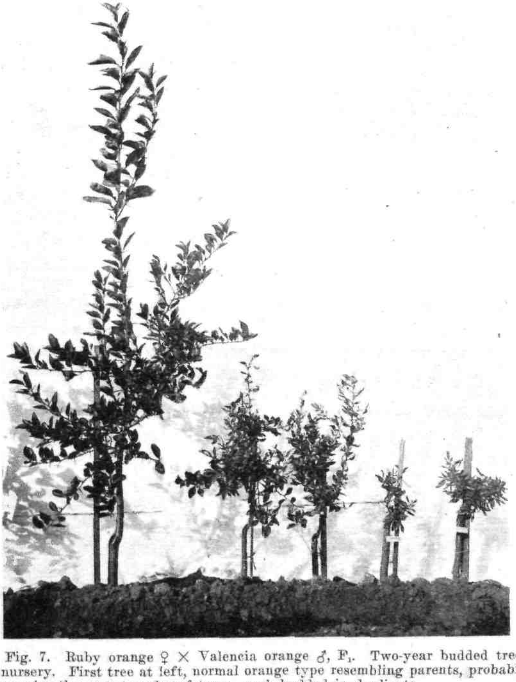

Fig. 7. Ruby orange ♀ × Valencia orange ♂, F_1. Two-year budded trees in nursery. First tree at left, normal orange type resembling parents, probably apogamic; the rest, two dwarf types, each budded in duplicate.

parental relationship. This heterotic effect probably increases in most cases to the limits of possible crossing, without being overcome by any unfavorable effect of the genic unlikeness of the parental forms.

Obviously, lethal and sublethal effects in selfing and crossing of Citrus may not be entirely the result of homozygosis of inevitably unfavorable genes, but may be in part a result of "incompatible" recombinations (Goodspeed and Clausen, 1917). In any case, the wide differences in vigor among progeny from the same parentage indicate complex heterozygosis of the parents.

When we consider the fertility of the F_1 hybrids, we find wide variability, similar to that with respect to vigor. The sterility shown by certain hybrids seems to be, in large part at least, a matter of individual genic composition. Thus Swingle's citrange evidence cited above indicates that these F_1 generic hybrids are sometimes highly fertile when selfed, and sometimes nearly or quite sterile (so far as viable embryos are concerned), aside from apogamic reproduction. This case therefore differs from that of certain Nicotiana species hybrids reported by East (1921), since the latter regularly show a high degree of sterility in the F_1 generation. This difference may well be due to a condition of complex heterozygosis in the Citrus species concerned.

The evidence so far discussed indicates that the apparent "breeding true" of selfed Citrus varieties, which naturally suggests homozygosis, is due primarily to a predominance of apogamic progeny, which seems to be usually much greater here than in species crosses. Further, probably many genes that come to expression in hybrids are usually or always suppressed in selfing. Finally, the appearance of uniformity is in part illusory, since, outside of special genetical cultures, occasional weak individuals are unlikely to come to fruiting, or to be noted at all without special search.

CHIMERAS AND BUD VARIATION

For centuries Citrus has been noted for striking somatic variations, especially for variant sectors in the rind of the fruit. Certain "bizzarria" forms, such as the one which Risso and Poiteau (1818–22, pl. 52) describe under the name "bigaradier bizarrerie," with fruits combining characters of two or three species, were attributed by two sixteenth-century writers, Porta and Nato (Savastano and Parrozzani, 1911), to development of sprouts from graft or bud unions.

Shamel and his associates (1912, 1918, 1918*a*, 1918*b*, 1920, 1920*a*, 1923, 1924, 1925) have shown that bud-variation forms in Citrus are

somewhat numerous and of considerable agricultural importance. Modern genetic theory provides three possible general explanations of the origin of such variations—gene or point mutation, chromosomal duplication and deficiency (whether involving whole chromosomes or limited sections), and loss and rearrangement of components in chimeras. Eyster (1924) has perhaps added a fourth in his hypothesis of *qualitative mitotic division of certain genes*. That is, while such a process would be included among the probably "diverse processes" (Sturtevant, 1925) of intragenic change, or mutation, of which we know so little, it seems to be essentially distinct from the fundamental changes which we surmise to supply the ultimate material of evolution. Eyster's intragenic units might, however, be merely labile "side-chains," *subordinate* elements in a single complex structure (the gene), and not the coördinate components of a compound genic structure.

R. A. Emerson (1922) has comprehensively discussed the origin and nature of bud variations. Coit (1915) has shown how chimeras may result from the occurrence of mutation in somatic tissues of Citrus. Clausen and Goodspeed (1923) have well presented some fundamental morphological considerations relating to chimeras, and pointed out the extreme difficulty of detecting the occurrence of gene mutation in such cases as that of Citrus.

It should be fairly easy to test the possibility of chromosomal mutation in forms that produce pollen, since Belling's (1921) iron-acetocarmine method can be used with Citrus (Longley, 1925; Frost, 1925, 1925*a*). The fact that bud-variation forms of Citrus often differ decidedly from the parent race in various characters, seems favorable to the possibility of chromosomal mutation. Little work seems to have been done anywhere which bears directly on the causation of bud variation in Citrus, although the evidence from other plants (Winkler, 1910; Bateson, 1916, 1919, 1921; Clausen and Goodspeed, 1923) suggests that chimeral phenomena may be of much importance.

If Citrus forms are extremely heterozygous, somatic gene mutations, if they occur, will relatively often come to somatic expression (Muller, 1918). Whether such heterozygosis favors abnormal somatic mitosis seems to be entirely in doubt. R. A. Emerson (1922) found that a variegation gene in maize "mutates" more frequently when combined with an allelomorph for white than when homozygous, and Eyster (1924) suggests an explanation based on his hypothesis of heterogeneous structure of the gene concerned. We might expect,

therefore, that the average instability of any such genes present in Citrus will tend to be increased in the presence of extensive heterozygosis.

A bud-variation type, whatever its cytological basis, presumably originates in a single cell. If the variation depends on non-disjunction, either of whole chromosomes or of smaller units of chromatin, twin daughter cells may carry two different and complementary new types (Eyster, 1925). If the variation involves some change occurring within a single gene according to the current conception of gene mutation in the narrower sense, a single new type is produced.

If a variant cell occurs and its descendants persist in the apical meristem of a shoot or bud, further development consists, for a time at least, of two kinds of tissue, and the shoot has become a chimera. Doubtless many variant initial cells are too deficient in vigor to compete successfully with normal cells, so that only a part of the new types formed ever come to dominate even one bud. Probably many twin variations are never recognizable as such, because of early elimination, either selective or accidental, of one of the two complementary types. This consideration increases the probability (R. A. Emerson, 1922) that unequal mitosis is the predominant cause of the origination of bud-variation types. It is interesting to note here that the bud-variation strains described by Shamel and his associates (1918, 1918*a*, 1918*b*, 1920, 1920*a*, 1923, 1924, 1925) seem generally to range from moderately less vigorous to considerably more vigorous than the parent variety.

At an early stage of the process described in the last two paragraphs, the shoot affected is an incomplete periclinal chimera, since the new type constitutes a sector in the cell layer or layers to whose formation the variant initial cell contributes. What happens later must depend on the spatial regularity and uniformity of the meristematic cell divisions, and on the location of new buds. There is reason to believe that Citrus chimeras are often relatively unstable in the relations of their components.

Sectorial fruit chimeras are frequent in Citrus (Coit, 1915; Babcock and Clausen, 1918; Shamel *et al.*, 1918, especially plates). Frequently a longitudinal sector differs from the rest of the fruit in thickness or color of rind. In some cases a whole tree shows so marked a tendency to the production of variant fruits that it appears to be in a chimeral condition throughout.

Sometimes a fruit has two adjacent sectors, of similar width, whose rind varies in opposite directions from the normal condition. This

may be considered definite evidence (Eyster, 1925) that the variation is due to differential mitosis, perhaps to non-disjunction of chromosomes. The paired sectors may be unlike either in color or in thickness of rind, or in both at once. If non-disjunction of whole chromosomes is not involved in all such cases, Eyster's hypothesis of qualitative division of individual genes may apply, in Citrus, to genes other than those especially determining color. In fact, there seems to be no reason to suppose that such a process, if it occurs at all, is confined to "color" genes, although it might seldom be discoverable in other cases.

The corrugated strain of navel orange described by Shamel *et al.* (1925) may be an unstable periclinal chimera. Coit (1915) has described a similar case.

A Valencia orange tree in one of our experimental plots has one large branch of a distinct type, which regularly produces seedless fruits with corrugated rind. If this branch is a chimera, it must be periclinal, and relatively stable. We have another form, derived from a variant branch of Valencia, selected by Shamel[8] for its corrugated rind, which seems to be a mixed chimera ("hyperchimera" of Winkler) of a peculiar kind. Some fruits are normal in appearance, but scattered among these are some which are completely and heavily corrugated. Many fruits are intermediate, ranging from nearly normal to much corrugated. In this case the usual visible variation among fruits is not, as in the case of the Golden Buckeye navel orange which is discussed in the next paragraph, in the relative superficial proportions of two separate components of the rind of the same fruit, but relates to the rind of the whole fruit. Some intermediate fruits, for example, have a smooth rind with broad, shallow ribs, while others show general but slight or moderate true corrugation. That this variation is not due to general physiological factors acting on a readily modifiable type, is indicated by the magnitude and generality of the variation, and is practically proved by one fruit which had sharply contrasting segments of normal and corrugated rind. We may surmise, therefore, that the intermediate fruits are periclinal chimeras in which the number of cell layers of the outer component varies, doubtless largely as a result of similar variation in the apical meristem of the young flower buds. Possibly, as Eyster (1924) assumes for variegation, an unstable gene is involved.

The case of the Golden Buckeye navel orange (Shamel *et al.*, 1925) is also of especial interest here. The rind of the fruit is more

[8] Mr. Shamel has kindly given me permission to publish this description.

yellowish and thinner than that of the Washington navel orange, with knobs, stripes, and wider sectors of thicker, rougher, more reddish rind, resembling that of Washington. Somewhat frequently, however, on our trees, a branch produces fruit having only the deeper, more reddish color of Washington.[9] In such cases the rind characteristics are like those of Washington; the "navel" is usually more conspicuous than in Buckeye; and the shape of the fruit changes, probably in part, at least, because of the greater development of the navel structure. The fruits on these variant branches seem, in fact, to be indistinguishable from those of Washington. Apparently the Golden Buckeye is a mixed or mosaic chimera, of which one component resembles Washington while the other is similar to the Golden Nugget (Shamel *et al.*, 1918). If it is a periclinal chimera, evidently the inner component must emerge with remarkable frequency. It may be worth noting here that the typical commercial Golden Nugget is a dwarf form, and that plantings of this variety are, according to Shamel, always mixed with standard-sized trees, possibly of bud-variation origin.

The Thomson orange (Shamel *et al.*, 1925) often gives rise to *several* other types of navel orange, and not simply to one type from which Thomson arose by bud variation. This case and other similar ones suggest that marked genic instability is an important factor in the situation with these forms. It does not seem likely that all these variations are produced by changes in chromosome number followed by chimeral phenomena, although some trees may well be complex chimeras.

Probably all of the three types of Citrus "bizzarria," described by Savastano and Parrozzani (1911) as natural hybrids, are chimeras, not interspecific hybrids. These authors mention frequent color chimeras in the fruits (see their plate 1). The great variation in sugar and acid, both between trees and on the same tree, shown by the only form extensively studied (orange-colored lemon, "limone aranciato"), plainly indicates a general chimeral condition, with the relationships of the two components decidedly variable. Forms like these, which clearly combine the characters of two or three species, are best explained by Porta and Nato's graftage hypothesis (p. 392). Chimeras due to graftage may be called *synthetic* chimeras. On the other hand, chimeras which arise as a result of genetic variation within a clone may be called *autogenous*.

[9] I have seen at least three or four such branches on two rather small trees.

Cavara (1912), after mentioning Savastano and Parrozzani's forms and their use of the word *chimera* for fruits of mixed type, described a tree with ribbed or corrugated fruits, which bore several branches with smooth-rinded fruit. He concluded that the tree was most probably a chimera resulting from graftage, but it may well have been an autogenous chimera.

Trees which merely produce occasional variant shoots or fruits may in some cases be periclinal chimeras throughout, like Winkler's (1910) solanaceous chimeras, and the apple chimera described by Stout (1921). The Citrus chimeras, however, are doubtless usually autogenous, while Winkler's forms, at least, are synthetic. Thus many of the observed instances of bud variation in Citrus may be merely the result of irregularities of growth in long-existent chimeras.

If chimeras are very common in Citrus, they may largely explain the genetic differences that occur among the apogamic progeny of the same parent tree (p. 379). Little "islands" of variant tissue, which might never come to dominate their respective branches, may often give rise to apogamic seedlings that are visibly unlike the parent.

SUMMARY

This paper reports experimental results bearing mainly on the genetic significance of apogamy in Citrus. It also attempts a general evaluation of the published evidence relating to Citrus genetics. The data and discussion may be summarized as follows:

1. Polyembryony occurs generally in Citrus; adventitious embryos develop by proliferation of cells surrounding the embryo sac. It is here shown that the embryos are often much more numerous than the resulting seedlings, and that horticultural varieties differ greatly in characteristic amount of apogamy. This last fact seems to be important in connection with the choice of clones for the production of nursery stocks.

2. Interspecific and intergeneric crosses involving Citrus species exhibit, aside from the apogamic progeny, remarkable variation in the F_1 generation, suggesting an extremely heterozygous genetic constitution in the parental forms. Most seedlings from selfing are closely similar to the parental clone.

3. Fertilization seems to be usually necessary for the initiation of apogamic development. The sexually produced embryo is, however, frequently eliminated by the competition of apogamic embryos. Data

here presented indicate that the sexual embryo is more often eliminated in clones in which apogamic embryos are especially abundant. This fact should be considered in planning Citrus hybridization.

4. Doubtless the elimination of sexual embryos is often highly selective, largely because of frequent development of homozygosis of unfavorable genes. It is to be anticipated, therefore that selective elimination in favor of the apogamic embryos will tend, in general, to be most severe with selfing, and least so in relatively wide crosses. Some evidence presented indicates that the sexual progeny from selfing usually are both fewer and weaker than those from crossing.

5. Some especially "wide" crosses show marked heterosis when the parental types, as represented by progeny produces asexually, are taken as the standard of comparison. By the proper standard (sexual progeny resulting from self-fertilization), with consideration of viability as well as of relative vigor of viable plants, the favorable effect of crossing is presumably general and great.

6. It is suggested that the occurrence of apogamy in Citrus has favored the development, perhaps by mutation, of a very complex condition of heterozygosis, probably including lethal and sublethal genes, in Citrus forms generally. Crossing may have produced or contributed to this result, but its agency need not be considered essential.

7. Bud variations apparently affecting whole branches are frequent in Citrus. Sectorial chimeras are common, and evidently periclinal and mixed chimeras also.

8. The *numerous bud-variation forms of Citrus presumably* originate in single cells, either by gene mutation or by differential mitosis. In the former case, at least, their somatic expression is doubtless favored by the presence of numerous heterozygous recessive genes. The production of recognizable bud variations then requires bud formation in an area of variant tissue, and may often be due to irregular tissue development in periclinal chimeras. The abundance of bud variations with some Citrus forms apparently depends upon a permanent chimeral condition of the types in question. Some of the bud variations of Citrus suggest a special genic instability, perhaps fundamentally unlike typical gene mutation, such as has been postulated for cases of variegation.

9. In addition to the *autogenous* chimeras just mentioned, *synthetic* chimeras, resulting from graftage, evidently occur in Citrus.

10. The remarkable variations which sometimes occur among apogamic seedlings may be partly due to chimeral conditions in the

parent trees. A pollen-sterile navel orange has produced apogamically several fertile non-navel progeny.

11. A "thick-leaved" apogamic form is described, which has been produced by four species and twelve horticultural varieties. It has been shown in two cases to be tetraploid, and presumably is so in general. It may be valuable as a means of producing triploid hybrids.

12. Some evidence is presented on seedling albinism; its frequent production by some parents may be due primarily to heterozygosis for various genes for albinism, and perhaps to the presence of unstable genes such as occur in various cases of variegation in other plants.

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